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Masitinib monotherapy in canine epitheliotropic lymphoma

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Abstract

This study evaluated efficacy and side effects of masitinib in canine epitheliotropic lymphoma. Complete remission occurred in 2 of 10 dogs and lasted for median 85 days. Five dogs went into partial remission for median 60.5 days. Three pretreated dogs did not respond to therapy. Side effects occurred in six dogs and were mostly mild to moderate. Immunohistochemistry was available for eight dogs. KIT receptor was negative in all of them, six of eight lymphomas stained strongly positive for stem cell factor (SCF). platelet-derived growth factor (PDGF)-AA was weakly positive in two and negative in six. PDGF-BB was negative in four tumours, weakly positive in one and strongly positive in three. One was strongly positive for PDGF receptor (PDGFR)-β, seven were negative for that receptor. Five showed strong expression of PDGFR-α, two showed weak expression, one was negative. In conclusion, masitinib is effective in treating canine epitheliotropic lymphoma. But its effects are most likely not generated through the KIT receptor.

Keywords
clinical pathology, oncology, small animal, tumour biology, tyrosine kinase

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Introduction

Canine epitheliotropic T-cell lymphoma (CETL) is a relatively rare disease and makes up 3–8% of all canine lymphoma and less than 1% of all canine skin tumours.[1,2] A standard of care has not yet been determined. In veterinary medicine, the most common therapeutic approach is systemic chemotherapy. Corticosteroids and Lomustine (CCNU) are the most commonly used agents, frequently combined with other cytotoxic drugs or L-asparaginase.[3–8] Results of the treatments have been variable.[9] Because of the high radiosensitivity of lymphatic cells, radiation therapy has also been used for the treatment of CETL. A case series with 14 dogs with mucocutaneous oral lymphomas treated with radiotherapy showed an overall response rate of 67%. Of these 14 dogs, 6 had T-cell epitheliotropic lymphoma.[10] Recent human studies have also shown promising results.[4,5]

Masitinib is a tyrosine kinase inhibitor (TKI) targeting KIT receptor and platelet-derived growth factor receptor (PDGFR). It is licensed for the treatment of canine mast-cell tumours and a number of studies have reported its efficacy mainly in dogs with mast-cell tumours.[11] Dubreil *et al.* demonstrated that masitinib is very selectively blocking KIT and PDGFR.[12] The same authors also showed that it has a good oral bioavailability and is usually well tolerated.[12] Apart from reversing chemoresistance, masitinib also had a direct mild antiproliferative effect on canine lymphoid cells *in vitro*.[13] A positive effect of masitinib in a dog with cutaneous T-cell lymphoma has been reported as well as in a few dogs with multicentric T-cell lymphoma (D Jagielski *et al.*, personal communication, 2011).

Considering the possible anticancer effect of masitinib in T-cell lymphoma and especially cutaneous T-cell lymphoma, the aim of this study

was to evaluate efficacy and side effects of masitinib in dogs with CETL.

Material and methods

Animals

Ten client-owned dogs with histologically diagnosed CETL were prospectively enrolled in this multicenter trial. The patients were presented between August 2009 and September 2011 in the oncology services of the following European clinics: Clinic of Small Animal Medicine, Centre for Clinical Veterinary Medicine, Faculty of Veterinary Medicine, LMU Munich, Germany; Hofheim Small Animal Clinic, Hofheim/Taunus, Germany; Department Clinical Sciences of Companion Animals, Utrecht University, The Netherlands; Small Animal Clinic, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany.

At their first visit, all dogs underwent a complete physical examination documenting all clinical cutaneous lesions. Staging included abdominal ultrasound, three-view thoracic radiographs, complete blood count (CBC), biochemistry and urinalysis including urine protein creatinine ratio (UP/C). Tumour diagnosis was always confirmed histologically. While on therapy, all dogs were presented every 3 weeks for a physical examination, CBC, biochemistry and urinalysis.

Treatment

Masitinib was administered orally by the owners at a target dosage of 12.5 mg kg^{-1} every day. Adverse events were recorded and graded according to the Veterinary Co-operative Oncology Group-Common terminology criteria for adverse events (VCOG-CTCAE) at each visit.[14] In case of an UP/C elevation over two or creatinine or blood urea nitrogen (BUN) 1.5 times higher than the upper reference range or albumin 0.75 times lower than the lower reference range, treatment was discontinued. If a dog developed signs of protein-losing syndrome (UP/C >2, albumin <0.75 lower limit of normal), therapy was interrupted until values had normalized to limit value. If the laboratory values deteriorated, again treatment was permanently discontinued. If hemolytic anaemia [haemoglobin (Hb) < 10 g μL^{-1} and bilirubin > 1.5 upper limit of normal] or anaemia with lack of regeneration (Hb < 10 g μL^{-1} and reticulocytes < 80 000 μL^{-1}) occurred, treatment was discontinued. In case of hepatic toxicity (alanine aminotransferase (ALT)/aspartate aminotransferase (AST) >3 upper limit of normal) or neutropenia (<2000 μL^{-1}), treatment was interrupted until normalization of values and then resumed at the same dose level. If these events occurred for a second time, treatment was interrupted until resolution and continued at a dose of 9 mg $kg^{-1}day^{-1}$. If laboratory changes occurred for a third time, a drug holiday was instituted followed by a further dose reduction to 6 mg $kg^{-1}day^{-1}$. If severe adverse reactions resumed even at this dose, treatment was permanently discontinued. If one of the above-mentioned adverse reactions and/or severe diarrhoea or vomiting persisted after dose reduction, treatment was also permanently discontinued.

Response

Clinical response was assessed every 3 weeks according to Response Evaluation Criteria in Solid Tumours (RECIST), modified for the evaluation of CETL.[15] If available, the three most dominant lesions were defined as target lesions. The longest diameter of each target lesion was measured using a calliper. The sum of these measurements was used for assessment of response.

Immunohistochemistry

Immunohistochemistry (IHC) for KIT, PDGF-AA (R&D Systems, Minneapolis, MN, USA), PDGF-BB, PDGFR-α, PDGFR-β and stem cell factor (SCF) (all Santa Cruz Biotechnology, Dallas, TX, USA) was performed on serial sections of routinely formalin fixed, paraffin embedded tumours from eight dogs after the clinical phase from tissue specimens taken prior to therapy. For two dogs, there was insufficient tissue for IHC testing. Deparaffinization and antigen retrieval were accomplished in the Dako PT Link (Dako, Carpenteria, CA, USA) using a high pH (SCF only) or low pH antigen retrieval solution (both Dako) for 20 min at 99 °C. Immunostaining was

performed on the Dako Autostainer Link 48 automated staining system (Dako) using a rabbit polyclonal anti-human KIT antibody (Dako), a goat polyclonal anti-rat PDGF-AA (R&D Systems Inc., Minneapolis, MN, USA), a rabbit polyclonal anti-human PDGF-BB, a rabbit polyclonal anti-human PDGFR-α, a rabbit polyclonal anti-human PDGFR-β and a mouse monoclonal anti-human SCF (all Santa Cruz Biotechnology) at a dilution of 1:100 followed by the Flex detection system with the appropriate secondary antibodies (Dako). The immunoreaction was visualized with 3,3-diaminobenzidine substrate (Dako) and sections were counterstained with haematoxylin. Positive immunohistochemical controls included mast-cell tumours and canine soft tissue sarcoma that had previously been shown to express the targets to which the appropriate antisera were added. For negative controls, the primary antibodies were replaced with homologous non-immune sera. Samples were scored based on the labelling intensity of neoplastic cells and percentage of positive neoplastic cells. Ultimately, tumours were scored as negative when there was no labelling in the examined sections or labelling was only observed in deeper tissue sections or less or equal to 20% of neoplastic cells were positive. Tumours with more than 20% strongly positive cells were scored as positive. A score of weak positive labelling was assigned to tumours that had at least 20% of neoplastic cells with weak positive labelling, but less than 20% of neoplastic cells expressing strong labelling.

Results

Ten dogs were enrolled in this study. The dogs' age ranged from 4 to 15 years, with a median of 10 years. There were two mixed breed dogs and eight pure breed dogs (one Australian shepherd, one German shepherd, one Belgian shepherd, one Picard, one West Highland white terrier, one Gordon setter, one Small Munsterlander and one Collie).

Six of the 10 dogs with CETL received masitinib as first-line therapy for their tumours. One dog was previously treated with a single dosage of steroids, one had been treated with CCNU and prednisolone for over 8 months, one dog had been given L-asparaginase, CCNU, doxorubicin and prednisolone and in one dog tumour nodules had been repeatedly surgically removed. In 5 of the 10 dogs with CETL, the tumour was generalized. In two dogs, the CETL was located on the lip, in one dog on the nose, the lip and the conjunctivas, in one dog nose, lips and vulva were affected and in one dog in the oral cavity with metastasis to the mandibular lymph node.

Two dogs went into complete remission (CR) and five dogs into partial remission (PR). In three dogs, masitinib was ineffective, and progressive disease (PD) was noted. In one dog, the CR lasted for 126 days. In another one, masitinib was discontinued after 1 year of treatment and the dog was still tumour free 3 years after diagnosis. In both animals, mastinib was the first-line therapy. In the five dogs with PR, median time to progression was 60.5 days (43 – 84 days). The overall response lasted for median 85 days. The three patients with PD had all been pretreated. One dog had been given a single injection of prednisolone, one had been treated with various chemotherapeutics as listed above and the third one underwent surgery prior to mastinib. Four of the five dogs with PR had untreated CETL before they received masitinib. The fifth dog was pretreated with CCNU and prednisolone. In addition to the seven dogs with tumour remissions on masitinib, one additional dog showed distinctive clinical improvement. This dog first went into PR and had PD 64 days after starting therapy. Despite having PD 64 days after starting therapy, the owner was pleased by the performance of the skin lesions as they were dry and not oozing or itching unlike their appearance before the masitinib treatment. The owner decided to continue administering the medication beyond the study end.

No side effects of masitinib were observed in 4 of the 10 dogs. As summarized in Table 1, the side effects noticed in the remaining six dogs were classified as mostly mild to moderate. An exception was one dog that developed grade 4 diarrhoea, grade 3 vomiting and anorexia as well as moderate hypoalbuminemia. This dog was euthanized due to diarrhoea.

Immunohistochemical examination for KIT, SCF, PDGFR-α/-β PDGF-AA/-BB was possible in 8 of the 10 cases. One (with PD) of these eight dogs was pretreated with a single injection of

Table 1. Side effects of masitinib in dogs with CETL treated with masinitib

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Anaemia (Hb ↓)	2/10	1/10	–	–	–
Neutropenia	1/10	–	–	–	–
Plt ↓	1/10	–	–	–	–
ALT ↑	–	–	1/10	–	–
AP ↑	–	1/10	–	–	–
Alb ↓	–	1/10	–	–	–
Creatinine	1/10	–	–	–	–
Anorexia	–	–	1/10	–	–
Vomiting	–	–	–	1/10	–
Diarrhoea	–	–	–	1/10	–
Weight loss	–	1/10	–	–	–
Petechiae	–	–	1/10	–	–

Alb, albumin; AP, alkaline phosphatase; Hb, haemoglobin; Plt, platelets.

prednisolone before biopsies were taken. The other seven dogs did not receive any chemotherapy or steroids before specimens were taken. In two dogs (one with CR, one with PD), no tumour specimens were available for immunohistochemical evaluation. It was initiated after the clinical phase to get a better understanding of the mechanisms of action of masitinib in CETL. Table 2 summarizes the results. All samples were negative for KIT. Three specimens stained negative for SCF (both PD dogs and one PR). In the other five cases, there was a strong positive reaction for SCF. PDGFR-α was tested negative in one dog with PR. The dog in CR as well as one in PR stained weakly positive for the receptor. Both dogs with PD and the remaining three in PR were strong positive. PDGFR-β was negative in all dogs except in one with PR. The specimens of three dogs with PR tested weakly positive for PDGF-AA. The other two PR samples as well as those of the two dogs with PD and the one in CR showed no expression of the growth factor.

Discussion

According to our study, the overall clinical response rate of 70% supports that masitinib has promising potential in treating CETL. This is in contrast to the observations by London *et al.* (2003) who used a different TKI (SU11654) in six dogs with CETL of which only one experienced a stable disease (SD).[16] The observed response rate in our study is comparable to that reported in studies using cytotoxic chemotherapy, in which measurable responses were achieved in 78 and 83%, respectively.[7,8] In the study of Williams *et al.* response to CCNU lasted for median 106 days while the median time to progression of 85 days in our study is similar to the results of Risbon *et al.* who reported a median response duration of 86 days.[7,8] Vail *et al.* treated nine dogs with

Table 2. Immunohistochemistry of CETL prior to treatment with masitinib

Response	Prior treatment	SCF	KIT	PDGF-AA	PDGF-BB	PDGFR-α	PDGFR-β
CR	None	Strong pos.	No labelling	No labelling	No labelling	Weak pos.	No labelling
PR	None	Strong pos.	No labelling	No labelling	Strong pos.	Strong pos.	Strong pos.
PR	None	Strong pos.	No labelling	Weak pos.	Strong pos.	Strong pos.	No labelling
PR	None	Strong pos.	No labelling	No labelling	No labelling	No labelling	No labelling
PR	None	Strong pos.	No labelling	No labelling	No labelling	Strong pos.	No labelling
PR	None	Strong pos.	No labelling	Weak pos.	No labelling	Weak pos.	No labelling
PD	Prednisolone (single injection)	No labelling	No labelling	No labelling	No labelling	Strong pos.	No labelling
PD	None	No labelling	No labelling	No labelling	Weak pos.	Strong pos.	No labelling

pos., positive.

CETL with pegylated liposomal doxorubicin, but with an overall response rate of 44%, this approach seems to be less effective than masitinib.[17] The study of Iwamoto *et al.* using linoleate to treat dogs with CETL showed clinical improvement and reduction or disappearance of lesions in six of eight dogs; however, there was no definition of CR and PR as well as stable and PD, so this study should be interpreted with caution.[18] Pegylated L-asparaginase used in seven dogs with CETL led to a median survival of 9 months but responses were only partial and often short lived.[3]

All except for one dog responding to masitinib did not receive any prior therapy and all three dogs with PD were pretreated, which might indicate that prior treatment may limit the efficacy of masitinib. Similar results are found in the study of Hahn *et al.*[11] It could not be determined if this was owing to changes in the expression of growth factor receptors and their growth factors because IHC was only performed on specimens taken prior to any therapy. In many neoplasms, it has been shown by various mechanisms cytotoxic drugs have the potential to either induce drug resistance or select for resistant subclones. Cancer cells can acquire a multidrug resistant (MDR) phenotype after exposition to one specific drug leading to cross-resistance to other structurally and functionally unrelated anticancer drugs.[19,20] In human leukaemia cell lines, it has been demonstrated that tumour cells can experience an up-regulation of MDR-1 as a result of treatment with a cytotoxic drug (e.g. an anthracycline) most likely resulting in cross-resistance to the TKI imatinib.[21] However, Zandvliet *et al.* have proven that masitinib can reverse doxorubicin resistance in canine lymphoid cell lines.[13] But no investigations have yet been made for the combined use of masitinib and doxorubicin *in vivo*. Several lines of evidence – predominantly in human medicine – have shown that besides acquired resistance to TKIs several malignancies could show a '*de novo*' resistance which leads to failure of a therapy that should have been effective according to the underlying biology and genetics of the tumour.[22] In the current study, no investigations have been made according to tumour kinase mutational status. In canine mast-cell tumours, most c-kit mutations have been identified in exon 11, but also in exons 7 and 8, as well as exon 17.[23,24] In human, gastrointestinal stromal tumours with KIT mutations in exon 11 have a higher response rate than those with exon 9 mutations or those without KIT or PDGFRA mutations.[25] Because all except for one specimen in this study showed weak to strong PDGFR-α expression, the observed responses could have been caused by their diverse mutational status.

Masitinib inhibits mainly the receptors KIT and PDGFR. Therefore, the immunohistopathological examination of the samples for these receptors and their corresponding factors should elucidate if these targets and their activating factors were present in the samples.

Activation of KIT is considered of paramount importance for proliferation, survival and differentiation of haematopoietic progenitor cells into mature cells. In human haematopoietic tumours, KIT is predominantly expressed by undifferentiated tumours of the progenitor cells and only rarely by those involving mature haematopoietic cells. These observations were recently confirmed in veterinary medicine by studies of Giantin *et al.* who evaluated KIT expression in canine lymphoma and leukemias. KIT mRNA reached significant levels only in some blastic (high grade) T-cell lymphomas and undifferentiated leukemias.[26] In dogs with CETL, significant KIT expression has only infrequently been reported.[27] In humans, KIT expression is present in only 30 – 50% of all cases of cutaneous lymphoma.[28] Another study examining 168 CD30+ lymphomas including 15 cutaneous anaplastic large-cell lymphomas found KIT expression to be exceedingly rare.[29] In this study, KIT receptor expression was found to be negative in all dogs tested. It is therefore concluded that KIT does not play a significant role in the pathogenesis of most canine CETL.

Besides inhibiting the KIT and PDGFRα/β receptors masitinib also targets other receptors and pathways such as lymphocyte-specific kinase (Lck), Lck/Yes-related protein (LYn), fibroblast growth factor receptor 3 (FGFR3) and focal adhesion kinase (FAK).[30] It is speculative if these pathways serve as targets in the masitinib effects on CETL. It is an interesting observation that in our study all except of one dog responding to masitinib reacted strongly positive for SCF, while SCF was negative

in the two dogs with PD. One dog with PD was pretreated with a single injection of prednisone before biopsies were taken. Therefore, it may be possible that SCF expression in this case was changed by the prior therapy which could have a negative impact on the efficacy of masitinib. SCF has the potential to synergize with other growth factors and thus gain influence on the phosphorylation of the EPO receptor and the beta chain of interleukin-3 receptor.[31–34] Lck together with other receptors has been shown in humans to play a role in apoptosis of lymphomas.[35] Further studies are needed to elucidate the impact of SCF and Lck as well as other non-KIT/non-PDGFR signalling pathways in the development of CETL and the possible effect masitinib may exert on these pathways.

PDGFR-β represents another potential target of masitinib but in our study showed strong expression in only one of eight dogs (PR). In all the other dogs, there was no or only very little PDGFR-β expression detected. It was expected that because of the inflammatory nature of epitheliotropic lymphomas, the expression level of PDGFR-β would be higher, because PDGFR-β can be upregulated by inflammatory or other stimuli while it is low in cells under physiological circumstances.[36] Based on our results, it has to be assumed that this receptor and its substrate do not play an important role in CETL, especially since the case with CR stained negative for both PDGF-BB and PDGFR-β.

PDGFR-α binds to all PDGF ligands and was found to be strongly positive in five dogs (three PR, two PD) and weakly positive in other two dogs (one CR, one PR). It is conceivable that masitinib may exert some effects on this receptor, but PDGF-α was also strongly positive in both dogs with PD which questions the impact of masitinib on the PDGFR-α pathway. A limitation of our study is that only two of three known PDGF receptors and two of five ligands were evaluated. Especially PDGF-AB was not evaluated in this study, although PDGF-AB can give stronger mitotic and chemotactic effects than PDGF-AA and -BB.[37,38] However, this effect occurs only in cells with the same amount of α- and β-receptors, which was only seen in one dog in which strong expression of both receptors could be demonstrated.

Besides measureable responses, palliation of tumour-associated symptoms is an important factor in veterinary medicine. One of the patients in this study developed PD after 2 month on masitinib, but the lesions remained dry and non-irritated with masitinib in contrast to prior treatment which led the owner to keep on administering masitinib. Fontaine *et al.* reported that 40% of dogs with CETL are presented with pruritus, and erosions and crusts occur in 60 and 46.6%, respectively.[39] In many cases, the clinical symptoms associated with the skin lesions are most irritating to the patient and may prompt owners to consider euthanasia. Therefore, patients may benefit from masitinib treatment by palliation of their clinical symptoms without tumour remission. Masitinib has also been proven effective in the treatment of canine atopic dermatitis.[40] Toceranib, another TK inhibitor approved for the use in dogs, has been reported to lead to clinical benefit in tumours that are often associated with inflammatory reactions (e.g. anal gland anal sac adenocarcinomas), poorly differentiated carcinoma (neck and jaw) and canine nasal adenocarcinoma.[41,42] It has been suggested that the antiangiogenic properties of toceranib from targeting VEGFR may be responsible for this effect, but the exact mechanisms of action on non-KIT-driven neoplasms are yet to be elucidated. Therefore, based on these preliminary observations, it can be hypothesized that TK inhibitors may exert mechanisms that target tumours with inflammatory components, such as CETL.

There were mainly only a few and mainly mild side effects which was to be expected based on the data of Hahn *et al.*[11] However, one dog with severe diarrhoea as a side effect of masitinib had to be euthanized. By combining masitinib with other established therapies like cytotoxic chemotherapy, surgery or radiation therapy, additive or synergistic effects may be achieved.[7–9,43–45] There are no published data in veterinary medicine on multimodality approaches to CETL which include TKIs. In human patients with epitheliotropic T-cell lymphoma, combined modality therapy has been shown to result in higher rates of complete responses.[46]

In conclusion, masitinib seems to have efficacy in CETL. From the limited data available, it appears

that in our cases inhibition of KIT and PDGFR did not play an important role in the clinical remissions observed. However, these preliminary results need to be interpreted with caution because of the limitations of this study, given the fact that only a heterogeneous, small and not placebo-controlled group of patients was treated. Therefore, further investigations are needed to substantiate our findings and elucidate the pathways by which masitinib acts against CETL.

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